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Award Number: DAMD17-00-1-0033

TITLE: Locating a Prostate Cancer Susceptibility Gene on the X Chromosome by Linkage Disequilibrium Mapping Using Three Founder Populations in Quebec and Switzerland

PRINCIPAL INVESTIGATOR: William D. Foulkes, M.B., Ph.D.

CONTRACTING ORGANIZATION: Montreal General Hospital Research Institute
Montreal, Quebec H3A 2T5
Canada

REPORT DATE: March 2005

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	March 2005	Annual (1 Mar 2004 - 28 Feb 2005)

4. TITLE AND SUBTITLE
Locating a Prostate Cancer Susceptibility Gene on the X Chromosome by Linkage Disequilibrium Mapping Using Three Founder Populations in Quebec and Switzerland

5. FUNDING NUMBERS
DAMD17-00-1-0033

6. AUTHOR(S)
William D. Foulkes, M.B., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Montreal General Hospital Research Institute
Montreal, Quebec H3A 2T5
Canada

E-Mail: william.foulkes@mcgill.ca

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)
At the Montreal site, 195 participants have consented to participate and their blood was drawn. We have another 15 men that have agreed to participate and their blood will be drawn in the next few months.We have recruited a total of 42 controls. The pedigrees for all controls and cases have been drawn. Ishihara charts were shown to all cases and controls and the results were recorded. At the Switzerland site, case ascertainment is underway. To date, an additional three urologist and a radio-oncologist have given their support to this project. 319 patients have been contacted and 102 have had a consultation with a DNA sampling. An additional 28 men have given their consent to participate at the Sion site. As well as continuing to look at the X chromosme for relevant markers, we have genotyped 11 microsatellite markers, spanning 34 megabases on chromosome 7, in approximately 140 of our cases. We observed suggestive differences between cases and controls for two markers located approximately 2.75 megabases apart at 7q11.23 (D7S2518 and D7S2204). We also conducted the first phase of a SNP discovery project. We re-sequenced CHEK2 in 75 Ashkenazi Jewish individuals (25 prostate cancer, 25 breast cancer and 25 controls). We identified 5 novel SNPs. These are now under detailed investigation. Finally, we completed and published our study of the putative prostate cancer gene, *MSR1*.

14. SUBJECT TERMS
No subject terms provided.

15. NUMBER OF PAGES
18

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Statement of Work

Task 1: Case ascertainment, contact, consent, interview, DNA extraction and pathology confirmation

- Obtain approval for this study from relevant IRBs

These goals have not been achieved at all sites. Human subject research cannot commence at the Chicoutimi site until the Army has granted approval. Please see the appendix for a full description on how we have worked to achieve this goal. In brief, in view of considerable and persistent obstacles to achieving our goals at this site, we have decided to abandon our efforts to recruit cases at the Chicoutimi site. We have obtained ethical approval at all the sites where we are currently conducting this study (Valais and McGill)

- Identify all prevalent cases of prostate cancer at hospitals serving the three populations under study: Chicoutimi, McGill University Hospitals (Quebec) and Sion and affiliated regional hospitals, Valais, Switzerland. This will be carried out by contacting medical records, out-patient charts and cancer registries, confirming that the patient is living and then seeking permission from treating physicians (who are collaborators on this proposal) to contact their patients by letter.

Montreal: This goal was achieved at the McGill University Hospitals last year and has been on-going this year. Thirty-nine additional cases were ascertained since March 2004.

Valais: In September 2004, we contacted Dr Sabine BIERI, head of the Service of Radio-oncology at the Hospital of Sion. She kindly accepted to actively collaborate to the project. She transmitted the names and administrative data of prostate cancer patients followed in the Service of Radio-oncology, who were willing to participate to a research project. The research nurse selected the eligible patients according to the lists established by the *Registre Valaisan des Tumeurs* and the Institute of pathology of the *Institut Central des Hôpitaux Valaisans* and a basic information sheet about the project was sent to these patients. Thus, the patients could contact the research nurse to get more information and, subsequently, confirm his participation. In case of no reply after 5 to 6 weeks, the research nurse had a direct contact by phone with the patient to get the decision re his participation to the project. Thanks to the collaboration of Dr S. BIERI, we already recruited a substantial number of patients (> 50 patients).

- Identify incident cases through urology clinics at the three centres (Chicotimi, McGill University, Sion). Method of contact as for prevalent cases: also, eligible individuals will be approached directly in the clinic.

Montreal: This goal has been achieved at the Montreal site. McGill Urology Associates are helping to identify new cases of prostate cancer.

Chicoutimi: As stated above, we have abandoned our efforts at this site.

Valais: Dr Daniel de WECK, head of the *Registre Valaisan des Tumeurs*, has previously established a list of all patients diagnosed with prostate cancer between 1997 and 2002 and who are residents in the *canton du Valais* (n = 730). Only patients whose surnames indicate an origin from the *canton du Valais* (based on the data from the *Association Valaisanne d'Etude Généalogique*) were conserved and thus considered as eligible for the study (n = 558). At the end of February 2004, a mailing was sent to the private general practionners in charge of the living patients. We proposed that they send an information sheet about the research project to their patients with the phone number of the research nurse to be contacted. To date, this mode of recruitment was not very efficient (< 20 patients). A prospective recruitment (new patients with diagnosis of prostate cancer) has also been encouraged, particularly from the 3 collaborating urologists.

- Contact relatives via case

Montreal: 42 controls have been recruited into the study at this site
Valais: There have been no controls recruited to date at this site.

- Consent all eligible participants (case n~640)

Montreal: A total of 195 patients at the McGill University Hospital sites have given their consent to participate. We have appointments to meet another 15 men that wish to participate in this study over the next few months. We have purchased DNA from Ashkenazi Jewish controls from Israel, as recruitment of controls was very slow in Montreal. A total of 18 affected men have refused to participate and we have found 7 of the ascertained are deceased.

Valais: Multiple personal contacts have been pursued with 3 urologists (all in private practice) in the canton du Valais (Drs N. DEFABIANI, H. PETER and C. BIEDERMANN). These physicians perform most of the prostatectomies both in private clinics and in the public regional hospitals in the canton du Valais. They all gave their agreement to participate to the study. With the help of the research nurse, they have contacted by mail most of their patients eligible for this research project.

130 cases at the Sion Hospital site have given their consent to participate. 1 person was excluded at the Sion site. recruited to date. The Sion research team has found getting access to familial controls is quite difficult and, in agreement with the principal investigator, Dr William Foulkes, the Sion team plans to collect non-familial age- and ethnically-matched controls from the blood donors' clinic in the Hospital of Sion and from a local familial medicine clinic.

- Interview and construct three-generation pedigree for each case and control

Montreal: A total of 195 pedigrees have been drawn for cases for McGill University Hospital site since the commencement of the study. We have also drawn 42 pedigrees for the controls that have participated in the study
Valais: A total of 102 pedigrees have been drawn for cases at the Sion site.

- Show Ishihara charts to cases

Ishihara charts have been shown to all participants and controls at all sites where the study is being conducted (McGill and Valais).

- Draw blood from all consenting participants

Blood has been drawn from all participants that have been consented at all sites (Montreal and Valais)

- Extract DNA locally at each participating centre, transfer aliquots of DNA to PI laboratory for quality check and storage

DNA has been extracted at the McGill University Hospital and the Switzerland site. We are waiting till more cases have participated before DNA from the Sion site is transferred to the PI laboratory.
We have also re-contacted 20 patients that have previously given a DNA sample because we have run out of their DNA sample or we do not have enough left to work with. Of those 20 contacted 7 have given another DNA sample and there remains 13 men that will come in at a later date to give another sample.

- Transfer representative slides and blocks to Montreal for central pathology review
  (*NB* this will take place after ascertainment as we expect few cases will be re-classified and subsequently excluded)

Slides and blocks from patients ascertained at the McGill University Hospital site have been transferred to a central pathologist for his review. The pathologist has completed his review of the material from 178 of the

participating patients. We now have a standard Gleason Score for 178 cases and the remaining blocks have been ordered to grade them also. However, we expect this task to be completed by the pathologist at the end of August 2005.

We will wait for more cases to be recruited at the Sion site before the central pathologist reviews them. It would be more cost efficient to transfer these in batches rather than a few at a time.

- Create central database at the Montreal General Hospital Research Institute

We have developed a database at the McGill University Hospital sites and it is continually updated as more cases and controls are recruited. At the present this database holds information on cases and controls from the Montreal site only but other data from other sites will be transferred when ready.

Tasks 2 and 3: Genotyping of DNA from cases and controls, followed by statistical analysis

Specific Aims for this reporting period:

1. To study *CHEK2* and its contribution to prostate cancer in the AJ population.
2. To identify a prostate cancer genotype / haplotype on chromosome 7q in the AJ population.
3. To validate *MSR1* as a prostate cancer susceptibility gene.

Studies and Results:

Project 1: *CHEK2* in the Ashkenazi Jewish Population

We have completed the first phase of this study. We sequenced 25 AJ prostate cancer cases and 25 controls. We detected 3 variants. One, S428F, was previously identified as a founder conferring a breast cancer risk in AJ patients. The other variants were uncharacterized. We evaluated the frequency of these variants by screening 135 additional prostate cancer cases and 200 additional AJ controls (from the National Laboratory for the Genetics of Israeli Populations) by SSCP or RFLP analysis, but found no difference in frequency between cases and controls for any of the variants. During screening, an additional variant, Y424H, was observed. We have now turned to our collaborators within the ICPCG to validate these variants in prostate cancer families and in a larger number of prostate cancer cases and controls. So far, the Y424H variant has been observed in several prostate cancer families.

Project 2: Chromosome 7 and prostate cancer in the Ashkenazi Jewish population

Recently, the prostate cancer research group from Fred Hutchinson, led by Elaine Ostrander, reported linkage to a large region of chromosome 7q in 36 Ashkenazi Jewish (AJ) families with several cases of prostate cancer. We tested 140 cases of AJ prostate cancer and 200 Israeli AJ controls (same as above) for 11 of the 16 markers used in the original report. We used the CLUMP program to compare allele frequency distributions between cases and controls and found suggestive differences at two markers, one of which was significant (D7S2518). We genotyped 7 additional markers near/between these 2 markers to extend our findings. Analysis of the new markers with CLUMP revealed no differences in allele frequency between cases and controls. We used the program PHASE (version 2.1, Stephens et al.) to reconstruct haplotypes from our genotype data and found that the region between the 2 original markers of interest showed suggestive, though non-significant, evidence of differences in haplotype distribution between cases and controls. However, the number and density of markers analyzed was insufficient for identification of a clear disease haplotype. A larger sample size and/or a greater number of markers would need to be analyzed to reach a definitive answer. Independent evidence from other ICPCG members studying the area suggests this is not an avenue worth pursuing. This work is complete.

Project 3: MSR1 999 C>T collaboration

Since Xu et al (2002) suggested MSR1 as a putative prostate cancer susceptibility gene, many studies have provided contradicting evidence about the implication of various MSR1 variants in prostate cancer. As part of an international collaborative effort to resolve this issue, 2943 men with invasive prostate cancer and 2870 controls were tested for 999 C>T (R293X), an MSR1 truncating variant. We contributed genotypes for 133 AJ prostate cancer cases and 133 AJ controls as well as for 293 individuals belonging to kindred previously recruited by the ACTANE consortium. The prevalence of mutation carriers did not differ between cases and controls, nor did it differ by country, ethnicity or source. This large-scale analysis found no evidence that MSR1 999C>T is associated with an increased risk of prostate cancer. This work is complete, and published ms is appended.

Significance:

Identification of variants in candidate genes in founder populations is an important step for validating their candidacy as prostate cancer susceptibility genes.

Task 4: Manuscript preparation

- Report major findings

Published paper (ARMY-FUNDED INDIVIDUALS IN BOLD, PI UNDERLINED):

Full paper

Hope Q, Bullock S, Evans C, Meitz J, **Hamel N**, Edwards SM, Severi G, Dearnaley D, Jhavar S, Southgate C, Falconer A, Dowe A, Muir K, Houlston RS, Engert JC, Roquis D, Sinnett D, Simard J, Heimdal K, Moller P, Maehle L, Badzioch M, Eeles RA, Easton DF, English DR, Southey MC, Hopper JL, Foulkes WD, Giles GG; The Cancer Research UK/British Association of Urological Surgeons' Section of Oncology Collaborators. Macrophage scavenger receptor 1 999C>T (R293X) mutation and risk of prostate cancer. Cancer Epidemiol Biomarkers Prev. 2005 Feb;14(2):397-402. (WDF is corresponding author)

Abstracts

Hamel N, **Kotar K**, Evans C, Engert J, Edwards S, Hope Q, Meitz J, Shun K, Eeles R, ACTANE collaborators, and the UK Familiar Prostate Cancer Study Coordinating group and collaborators, Easton D, Giles GG, Hopper JL, Foulkes WD. Candidate genes in prostate cancer: a case-control approach. British Society of Human Genetics Annual Meeting 2004, York, England. Poster presentation (paper published, see above)

Hamel N, **Kotar K**, **Chen LQ**, Greenwood C, Foulkes WD. Preliminary evidence for a prostate cancer susceptibility gene on chromosome 7q using unselected Ashkenazi Jewish prostate cancer cases. American Society of Human Genetics 2004 Annual Meeting, Toronto; Poster 531. (paper now in preparation)

(see attached ms)

Appendix 1

Log of activities at the McGill University,Chicoutimi and Sion sites.

Abbreviations:

JGH-Sir M.B.Davis-Jewish General Hospital
MGH-Montreal General Hospital
RVH-Royal Victoria Hospital
MUHC-McGill University Health Centre
MGHRI-Montreal General Hospital Research Institute

Through out the year we continued towards getting ethical approval and completing the SPA application at the Chicoutimi site. Throughout the whole process of completing the SPA application we seemed to have had either personnel leaving that region or ethical approval had lapsed. For the past three years we have had the collaborating physician leave the region(Dr Bellevance) and had to wait some time before another would agree to the collaboration(Dr Paradis). This caused all of our pervious ethics documents and SPA application documents to need amending.

↓

New documents were submitted with the new collaborator's name on them and then we learned that the names of the IRB committee had changed. New documents had to be obtained(i.e the C.V of the new temporary Chair of the IRB at the Chicuotimi site) and then translated for the SPA application. However Chicoutim was having a hard time finding a permanent Chair –person for their IRB and every time we asked for their C.V we were told it was an interim person that would be filling the spot and a permanent Chair had not been found, consequently the IRB meeting were few and far between. At the same time the research team in Chicoutimi was having difficulty filling certain required documents for the SPA application such as the " Question for foreign institutions". Chicoutimi is a relatively small hospital centre in a very rural part of Quebec that is not accustomed to filling out SPA application related documents and they became increasingly frustrated with all the back and froth and having to re-obtain the same documents. Because Chicoutimi is approximately 500 miles away, it was very difficult for our team to obtain the required documents and we decided to concentrate our efforts at the sites where we had ethical approval(**Montreal,Valais**)

↓

Regular meeting have been attended with different persons involved in the project at the Sion site and continue to be on their agenda.

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In July 2004, we continued to request IRB re-approval for the project from the MUHC, JGH, MGH, RVH. We have approval at all Montreal sites.

↓

14 cases have donated blood and answered questions about their family medical history. Ishihara charts were shown to all these patients and the results were recorded. A total of

14 pedigrees were drawn for these cases.(March 2004-February 2005). All cases previously recruited were contacted for a follow up to update our database and patient information(approx. 110 cases).

↓

Due to the difficulty we have had in recruiting controls into the study we have decided to buy DNA from controls in Isreal.

↓

In 2004 we continued to contact tumor registries at the MUHC sites to obtain updated lists of patients who had been diagnosed with prostate cancer. An additional 39 cases were ascertained between March 2004 and February 2005.

↓

Pathology material for 178 cases at the MUHC were received and reviewed by a pathologist(Dr L Begin) to confirm the diagnosis of prostate cancer and to obtain a standard Gleason score among all cases. We now have Gleason scores for all 178 cases examined by Dr Begin.

↓

We obtained a list of patients wit a recent diagnosis of invasive prostate cancer(05-2002-05-2003) at the Sion site. Four urologists agreed to send a letter to their respective eligible patients. 558 affected men were ascertained from the Bern tumor registry and are being contacted by their physicians and the research nurse.(03-2003-03-2004)

↓

To date, 319 patients were invited to participate. 130 patients have agreed to participate in the study; blood sampling and DNA extraction is currently being carried out for these patients. One patient was excluded from the study.

↓

At the MUHC, another 15 patients have agreed to participate in the study and we have arranged to meet most over the next three months. We anticipate more controls participating but not a large number. We are actively working to recruit more cases at all sites where we have approval.

↓

We have continued to examine prostate cancer families and hope to look more closely at familial syndromes for prostate cancer. To date we have ascertained four prostate cancer families into our study.

↓

Genotyping of 11 markers on chromosome 7 to narrow down the region of linkage. Results confirmed that a potential area of linkage does exist in the vicinity of markers D7S2518 and D7S2204, but revealed that our markers were too few and too far apart to identify a clear disease haplotype from our data. We genotyped our cases and controls for 7 additional markers. Analysis of the region between D7S2518 and D7S2204 (but not including these markers) showed suggestive evidence of differences in haplotype distribution between cases and controls.

↓

We have begun CHEK2: 1100delC mutation analysis in cases and controls but we have only preliminary data up to this point, we have now commenced a SNP discovery project (March 2004-February 2005)

Macrophage Scavenger Receptor 1 *999C>T* (R293X) Mutation and Risk of Prostate Cancer

Questa Hope,[1] Sarah Bullock,[1] Christopher Evans,[3] Julia Meitz,[1] Nancy Hamel,[4] Stephen M. Edwards,[1] Gianluca Severi,[9,15] David Dearnaley,[1] Sameer Jhavar,[1] Christine Southgate,[1] Alison Falconer,[1] Anna Dowe,[1] Kenneth Muir,[10] Richard S. Houlston,[2] James C. Engert,[6] David Roquis,[7] Daniel Sinnett,[8] Jacques Simard,[11] Ketil Heimdal,[12] Pål Møller,[12] Lovise Maehle,[12] Michael Badzioch,[13] Rosalind A. Eeles,[1] Douglas F. Easton,[3] Dallas R. English,[15] Melissa C. Southey,[14] John L. Hopper,[14] William D. Foulkes,[5] Graham G. Giles,[15] and The Cancer Research UK/British Association of Urological Surgeons' Section of Oncology Collaborators

[1]Translational Cancer Genetics Team, Section of Cancer Genetics and Urology Unit, Male Urological Centre and Institute of Cancer Research and Royal Marsden NHS Trust; [2]Molecular and Population Genetics Team, Section of Cancer Genetics, Institute of Cancer Research, Surrey, United Kingdom; [3]Cancer Research UK Genetic Epidemiology Unit, Strangeways Laboratories, University of Cambridge, Cambridge, United Kingdom; [4]Research Institute of the McGill University Health Centre, [5]Program in Cancer Genetics, Departments of Oncology and Human Genetics, and [6]Mike Rosenbloom Laboratory for Cardiovascular Research, Division of Cardiology, Faculty of Medicine, McGill University; [7]Centre d'Innovation Génome Québec et Université McGill, Plate-forme Sequencage; [8]Centre de Cancérologie Charles Bruneau, Centre de Recherche de l'Hôpital Sainte-Justine, Département de Pédiatrie, Université de Montréal, Montréal, Québec, Canada; [9]Division of Epidemiology and Biostatistics, European Institute of Oncology, Milan, Italy; [10]Division of Epidemiology and Public Health, University of Nottingham, Nottingham, United Kingdom; [11]Cancer Genomics Laboratory, CHUL Research Centre, Sainte-Foy, Quebec, Canada; [12]Section of Genetic Counselling, Department of Cancer Genetics, Norwegian Radium Hospital, Oslo, Norway; [13]Department of Medicine, Division of Medical Genetics, University of Washington, Seattle, Washington; [14]Centre for Genetic Epidemiology, Department of Public Health, University of Melbourne; and [15]Cancer Epidemiology Centre, The Cancer Council Victoria, Carlton, Victoria, Australia

Abstract

Background: **Variants in the gene encoding the macrophage scavenger receptor 1 (MSR1[4]) protein have been identified in men with prostate cancer, and several small studies have suggested that the *999C>T* (R293X) protein-truncating mutation may be associated with an increased risk for this disease.**
Methods: **Using large case-control, cohort, and prostate cancer family studies conducted in several Western countries, we tested for the *999C>T* mutation in 2,943 men with invasive prostate carcinoma, including 401 males from multiple-case families, 1,982 cases unselected for age, and 575 men diagnosed before the age of 56 years, and in 2,870 male controls. Risk ratios were estimated by unconditional logistic regression adjusting for country and by a modified segregation analysis. A meta-analysis was conducted pooling our data with published data.**
Results: **The prevalence of *MSR1*999C>T* mutation carriers was 0.027 (SE, 0.003) in cases and 0.022 (SE, 0.002) in controls, and did not differ by country, ethnicity, or source. The adjusted risk ratio for prostate cancer associated with being a *999C>T* carrier was 1.31 [95% confidence interval (CI), 0.93-1.84; $P = 0.16$]. The modified segregation analysis estimated the risk ratio to be 1.20 (95% CI, 0.87-1.66; $P = 0.16$). The risk ratio estimated from the meta-analysis was 1.34 (95% CI, 0.94-1.89; $P = 0.10$).**
Conclusion: **Our large-scale analysis of case and controls from several countries found no evidence that the *999C>T* mutation is associated with increased risk of prostate cancer. The meta-analysis suggests it is unlikely that this mutation confers more than a 2-fold increased risk.** (Cancer Epidemiol Biomarkers Prev 2005;14(2):397–402)

Introduction

Many studies have shown that prostate cancer exhibits familial aggregation, but the genetic and shared nongenetic factors underlying the familial risk are unknown (1-3). Many studies have claimed linkage of prostate cancer to different genomic regions, but as yet no genes that are mutated in a significant proportion of multiple-case prostate cancer families have been conclusively identified (4, 5). It is possible that many loci with a wide spectrum of risks and different modes of inheritance are involved in genetic susceptibility to prostate cancer (2, 6).

Linkage to a region on chromosome 8p has been reported from analysis of 159 multiple-case families (heterogeneity logarithm of odds score, 1.84; $P = 0.004$; ref. 7) and has also been reported in a Swedish study (8). Subsequently, it was reported that variants in the gene encoding the macrophage scavenger receptor 1 (*MSR1*), situated within the linked region on 8p, were more frequent in men with prostate cancer than in controls (9). These variants included six rare missense mutations and one nonsense mutation, *999C>T*. The latter variant, numbered according to GenBank accession number NM_138715, is predicted to result in a truncated protein (R293X) in such a fashion that the region that is critical for ligand binding would be lost. Initially, this protein-truncating mutation was detected in 8 of 317 (0.025) men with prostate cancer not meeting the clinical criteria for hereditary prostate cancer, but in only 1 (0.004) of 256 unaffected men, yielding a

Received 3/15/04; revised 7/6/04; accepted 9/3/04.

Grant support: U.S. Army grant DAMD17-00-1-0033 and the Canadian Genetic Diseases Network (W.D. Foulkes); NIH grant U01 CA89600 (W.D. Foulkes, R.A. Eeles, G.G. Giles, D.F. Easton, and J.L. Hopper), The Prostate Cancer Charitable Trust, Times Christmas Appeal, Institute of Cancer Research, Cancer Research UK, BREAKTHROUGH Breast Cancer, and a legacy of the late Marion Silcock (R.A. Eeles); National Health and Medical Research Council grant 126402 (J.L. Hopper); Tattersall's and the Whitten Foundation, the Cancer Council Victoria, National Health and Medical Research Council grant 20905, VicHealth grant 1999-0227 (J.L. Hopper and G.G. Giles); and Fonds de la recherche en Santé du Québéc Network of Applied Genetic Medicine (D. Sinnett and J. Simard).

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Note: Q. Hope, S. Bullock, and C. Evans contributed equally to this work. J. Simard holds a Canada Research Chair in Oncogenetics and D.F. Easton is a Principal Research Fellow of Cancer Research UK. K. Heimdal is currently in the Department of Medical Genetics, Rikshopitalet, University Hospital, Oslo, Norway. The list of Cancer Research UK/British Association of Urological Surgeons' Section of Oncology Collaborators are available on request.

Requests for reprints: William D. Foulkes, Program in Cancer Genetics, Room L10-120, Montreal General Hospital, 1650 Cedar Avenue, Montreal, Quebec, Canada H3G 1A4. Phone: 514-934-1934-44121; Fax: 1-514-934-8273. E-mail: william.foulkes@mcgill.ca

crude odds ratio of 6.6 [95% confidence interval (CI), 0.9-294; $P = 0.08$]. Among the hereditary prostate cancer families, the heterogeneity logarithm of odds for linkage to this region was 1.40 in families carrying a variant in *MSR1* compared with 0.05 in those without, although families carrying the *999C>T* mutation did not show formal evidence of linkage ($P = 0.3$; ref. 9). An increased prevalence of variants in cases was also found in a study of African Americans (10). Since then, a study of 438 affected males from families with multiple-case prostate cancer, 492 unselected prostate cancer cases, and 488 controls found the frequency of *999C>T* mutation carriers to be 0.017, 0.028, and 0.033, respectively, providing no evidence for an increased risk (11). A further study from Finland also found no association between the R293X mutation and risk of prostate in cancer in either a hereditary or sporadic setting (12). To definitively confirm or refute the proposed association, we have genotyped the *999C>T* (R293X) variant in large case-control, cohort, and prostate cancer family studies conducted in several Western countries.

Methods

Subjects

Australia: Case-Control Study. Eligible cases were residents of Melbourne, Sydney, and Perth and identified from the population cancer registries, with histopathologically confirmed prostate cancer, excluding tumors with Gleason scores of <5, diagnosed before the age of 70 years with sampling stratified by age at diagnosis. Eligible controls were males identified through government electoral rolls and frequency matched to the age distribution of the cases (i.e., there is no statistical difference in the ages of cases and controls; refs. 13-15). For this study, 827 of the 832 cases and all of the 735 controls from whom blood was sampled were genotyped. The self-reported ethnicity of the cases and controls was 97% Caucasian.

Australia: Cohort Study. Cases and controls were identified from a prospective cohort study of 17,154 men ages 40 to 69 years at recruitment in 1990-1994 (16). For this study, all 469 incident cases identified up to June 2002 and 1,637 randomly selected controls were genotyped. The self-reported ethnicity of the cases and controls was 98% Caucasian (mean age, 61.8 years; range, 40-69 years). The controls were a random sample of all men in the cohort (mean age, 54.8 years; range, 40-69 years).

Australia: Early-Onset Case Series. Eligible cases were all men with histopathologically confirmed prostate cancer diagnosed before the age of 56 years identified from the Victorian Cancer Registry since 1999. For this study, 318 of 357 cases were genotyped. The self-reported ethnicity of the cases was 98% Caucasian.

United Kingdom: Unselected Case Series. Eligible cases were all UK-born men diagnosed with prostate cancer who attended the Urology Unit of the Royal Marsden NHS Trust, London, United Kingdom. For this study, 631 of 635 cases were genotyped. Twenty-one of these cases were also enrolled in the early-onset case-control study and are included in that study for the purposes of this analysis. The analysis was therefore based on 610 cases. Cases with known self-reported Afro-Caribbean origins were excluded because the UK controls were largely or entirely of White ethnic origins. The mean age of the cases was 69.0 (SD, 6.40).

United Kingdom: Early-Onset Case-Control Study. Eligible cases were all men with histopathologically confirmed prostate cancer diagnosed before the age of 56 years referred to a national study through collaborating general practitioners (mean age, 50.7 years; SD 3.90; ref. 17). Male controls were identified through the case's general practitioner, but not all cases could be matched. The controls were matched to the cases on age, and the only exclusion criterion was a previous diagnosis of prostate cancer. For this study, 259 of 267 cases diagnosed before the age of 56 and 186 of 189 controls available were genotyped. Two affected males also included in the ACTANE consortium set were also included in the latter study for the purposes of analysis.

United Kingdom: Spouse Controls. Eligible controls were the UK-born male spouses ($n = 147$) of cases enrolled in a UK population–based study of colorectal cancer (principal investigator, RSH). For this study, 141 males were genotyped. No ethnicity data were available. The mean age of these controls was 53.0 years (SD, 8.51).

Canada: Ashkenazi Jewish Case-Control Study. Eligible cases were prevalent invasive prostate cancers diagnosed in Ashkenazi Jewish men attending McGill University teaching hospitals in Montreal, Canada (18). For this study, 133 of 145 cases were genotyped. Eligible controls were men of Ashkenazi Jewish origin obtained anonymously from a genetic study of Quebec populations. For this study, 133 men were genotyped. The mean age at diagnosis of cases was 67.6 years (SD, 7.38). The ages of the controls are unavailable as they were anonymous at ascertainment.

The ACTANE Consortium: Multiple-Case Prostate Cancer Families

Multiple-Case Familial Prostate Cancer Series. Eligible subjects were males whose DNA samples were obtained because they belonged to families that had three or more cases of prostate cancer diagnosed at any age. The ascertainment of families with multiple cases of prostate cancer by an international consortium has been described previously in the context of a genome-wide search (19), wherein 65 families with the highest e-logarithm of odds scores were included. For this study, 180 affected (cases) and 34 unaffected males from 65 prostate cancer families were genotyped. The countries of origin of these families are shown in Table 1.

Familial Prostate Cancer Series. Eligible subjects, ascertained in Montreal, were males whose DNA samples were analyzed from Canadian and U.S. families that had multiple cases of prostate cancer but were not included in the above-mentioned genome-wide search. For this analysis, 229 affected males (cases) and 4 unaffected males (controls) from 156 families were genotyped. The ethnic origin of these cases was mixed White European.

Laboratory Methods. The *999C>T* mutation was analyzed either by restriction digestion analysis or by direct sequencing. One of four sets of primers was used to amplify the exon 6 region surrounding the variant (details of the primer sequences and conditions used are available from the corresponding author). When using restriction analysis as an analytic method, the PCR product was digested with the *Nla*III restriction endonuclease (New England Biolabs, Beverly, MA) according to the manufacturer's instructions to generate smaller digested fragments in the presence of the *999C>T* mutation. Digested products were visualized by agarose gel electrophoresis. Mutation carriers from the UK and Australian sets were confirmed by metaphor agarose gel electrophoresis (BioWhittaker, Walkersville, MD). A selection of UK and Australian samples heterozygous and homozygous for the wild-type alleles (as well as the single Australian sample that was homozygous for the rare allele) was also confirmed by sequencing. A subset of samples including all genotypes observed was analyzed with both methods for concordance: there were no discordant results. Comparative resequencing was done on PCR products purified with Multiscreen PCR 96-well plates (Millipore, Billerica, MA; Montreal samples).

Sequencing reactions were done using the ABI Prism dRhodamine Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA; UK samples) or the BigDye Terminator Cycle sequencing ready reactions kit (version 2.0, Montreal samples; Applied Biosystems, Foster City, CA). The products were analyzed on an ABI 377 Genetic Analyzer (UK samples) or on ABI 3700 automated DNA sequencers (Montreal samples; Applied BioSystems). The run files were processed using Sequencing Analysis software (version 3.6) and then aligned and compared using either Autoassembler 2.1 (Applied BioSystems) or PHRED and PHRAP (Montreal samples).

Statistical Methods. The association between the *MSR1*999C>T* mutation and prostate cancer was assessed using two methods. First, the estimated prostate cancer risk ratio associated with being a *MSR1*999C>T* carrier was estimated using logistic regression. To allow for potential differences in allele frequency among populations, the analysis was stratified by country (United Kingdom, Australia, Canada, Norway, and United States). Analyses were done using STATA version 7.0. To account for the fact that some individuals were related, 95% CIs for the risk ratio were computed using the Huber-White sandwich estimator, with the robust option in Stata.

Although the logistic regression analysis provides a valid test of the null hypothesis of no association, the estimate of the risk ratio is biased away from 1 because some cases were ascertained on the basis of having a family history. To adjust for this bias, the data were also analyzed by a modified segregation analysis (20) under a model described in terms of the risk ratio r and the allele frequencies p_k ($k = 1, \ldots, 3$). The incidence rates in country k (Australia, United Kingdom, or other) at age t were assumed to be $\lambda_0(t,c)$ in noncarriers and $r\lambda_0(t,c)$ in carriers. The rates $\lambda_0(t,c)$ were chosen at each stage so that the overall prostate cancer age-specific incidence rates agreed with national rates for the period 1988-1992 (21). This model was implemented using the pedigree analysis program MENDEL (22, 23).

A meta-analysis of our data combined with all other published data on the *MSR1*999C>T* mutation and risk of prostate cancer was conducted. Studies were identified by Medline searches using the terms MSR1, macrophage scavenger receptor, and prostate cancer. Where possible, we divided publications into their component substudies. Thus, there are three case-control series from one study (9) and three from the current study. The meta-analysis was conducted using the S-plus 6.1 statistical software package (Insightful Corp., Seattle, WA), using standard methods for combining the crude estimates of odds ratios based on the weighted sum of the log estimates with the inverse of the variance of the estimate as weight (24). The odds ratios for the different studies, with exact 95% CIs and P values, were calculated by using the StatXact statistical software package v.4.0.1 (Cytel Software Corp., Cambridge, MA).

Homogeneity in risk ratios across studies was evaluated by calculating the weighted (inverse of variance) sum of the squared differences between the log risk ratio estimates and the log of the pooled risk ratio estimate and assuming that this statistic follows a χ^2 distribution with $n - 1$ degrees of freedom (where n = number of studies). Homogeneity in the genotype frequencies across studies was tested separately for cases and controls by using a χ^2 test.

Results

The overall prevalence of *999C>T* mutation carriers was 0.027 (SE, 0.003) in cases and 0.022 (SE, 0.002) in controls. These prevalences did not differ by country (P = 0.6 and 1.0, respectively), ethnicity (P = 0.7 and 0.9, respectively), or source of subjects (P = 0.9 and 1.0, respectively). Moreover, the prevalence was similar in the UK cases <56 years old and spouse controls (P = 0.5) and in the controls from the Australian cohort and case-control studies (P = 0.9; Table 1). After adjusting for country, the estimated risk ratio from the logistic regression analysis was 1.31 (95% CI, 0.93-1.84; P = 0.12). The modified segregation analysis estimated the risk ratio to be 1.20 (95% CI, 0.87-1.66; P = 0.27; Table 2).

The logistic regression analysis showed no support for an association between the *999C>T* mutation and prostate cancer within either the UK or Australian samples (Table 2). Using the modified segregation analysis in which the country-specific population rates are taken into consideration, the risk ratio estimated from the Australian data was 1.48 (95% CI, 0.98-2.21; P = 0.06), but this was not different from the combined estimate from the other studies of 0.88 (95% CI, 0.46-1.67; P = 0.7).

Table 1. *MSR1*999C>T* mutation status for cases of prostate cancer and controls by study design

Study	Prostate cancer cases			Controls		
	Carrier	Wild-type	%	Carrier	Wild-type	%
Unselected series						
Australian case-control study	27	800	3.3	17	718	2.3
Australian cohort study	12	457	2.6	33	1,604	2.0
Australian<55 y series	8	310	2.5			—
UK systematic series	14	596*	2.3			—
UK <56 y series	6	251*,†	2.3	5	181	2.7
UK spouse controls			—	3	138	2.1
Canadian Ashkenazi Jewish	4	129	3.0	3	130	2.3
Multiple-case prostate cancer kindreds						
Australia	4	67	5.6	1	18	5.3
Canada	0	54	0.0			—
United Kingdom	0	41†	0.0	0	13	0.0
United States	1	9	10.0	0	1	0.0
Norway	0	4	0.0	0	1	0.0
Other prostate cancer kindreds						
Canada	1	107	0.9	0	1	0.0
United States	3	118	2.5	0	3	0.0
All United Kingdom	20	888	2.2	8	332	2.4
All Australian	51	1,634	3.0	51	2,340	2.1
All	80	2,943	2.7	62	2,808	2.2

*Twenty-one cases in common are classified with the UK<56 series.

†Two cases are classified with the multiple-case prostate cancer kindreds. There is one homozygote control in the Australian cohort study.

Table 2. Estimates of prostate cancer risk associated with being a *MSR1*999C>T* mutation carrier

	Logistic regression, OR (95% CI)	Modified segregation analysis	
		RR (95% CI)	Allele frequency (95% CI)
United Kingdom	1.16 (0.52, 2.57)	1.05 (0.43, 2.57)	0.010 (0.002, 0.019)
Australia	1.37 (0.93, 2.02)	1.48 (0.43, 2.57)	0.010 (0.007, 0.014)
Other	1.07 (0.29, 4.02)	0.72 (0.28, 1.82)	0.016 (0.000, 0.031)
All	1.31 (0.93, 1.84)	1.20 (0.87, 1.66)	0.011 (0.008, 0.014)

Abbreviations: OR, odds ratio; RR, relative risk.

Table 3 and Figure 1 show the point estimates for the prevalence of mutation carriers in cases and controls and the subsequent risk ratio estimates for association with prostate cancer, based on combining our data with that from others identified within published studies. The pooled estimate was 1.34 (95% CI, 0.94-1.89; $P = 0.10$). There was no evidence for heterogeneity in risk ratios ($P = 0.3$), although there was evidence for heterogeneity of the frequency of mutation carriers among controls ($P = 0.0004$) but not among cases ($P = 0.2$). The meta-analysis does not suggest that there is a statistically significant excess of *MSR1*999C>T* carriers in cases compared with controls. Moreover, when the study of Xu et al. (9) that generated the initial hypothesis is excluded, the risk ratio decreases to 1.25 (95% CI, 0.88-1.78).

Discussion

We have attempted to replicate the findings of the initial, and much smaller, study that found evidence for a strong association between the *MSR1*999C>T* mutation and risk of prostate cancer (9). We conducted an analysis on nearly 3,000 cases and over 2,800 controls from several countries and did a pooled analysis that combined our data with that identified in published studies to date. To our knowledge, this is the largest single allelic association study of prostate cancer. We found that there was no significant difference in the frequency of the *MSR1*999C>T* mutation between cases and controls, although the results from the Australian study almost achieve statistical significance (risk ratio, 1.48; 95% CI, 0.98-2.21; $P = 0.06$). The meta-analysis did not indicate that the *MSR1*999C>T* mutation is more prevalent in prostate cancer cases compared with controls. Overall, both our new analyses and those of the meta-analysis suggest that if the mutation is associated with an increased risk of prostate cancer, its effect on risk is not large and is unlikely to be more than 2-fold, as judged by the upper 95% limits of the confidence intervals.

Although it provides a valid test of the association, the risk ratio estimates from the standard case-control analysis using logistic regression are theoretically biased away from unity because some cases (but not controls) were selected on the basis of having a family history of prostate cancer. This bias, which would affect other similar studies, can be avoided by use of a segregation analysis approach that allows the family history of prostate cancer and genetic relationships between individuals to be taken into account naturally. In the analyses presented here, we used a simplified version that ignores other causes of familial aggregation of prostate cancer. This simplifying assumption is approximately equivalent to assuming that *MSR1*999C>T* and other risk factors combine multiplicatively (20). The risk ratio estimate from this approach (1.20) was slightly lower than that from the logistic regression approach (1.31).

The *MSR1* gene was initially studied in the context of prostate cancer because of suggestive linkage to the region in which this gene lies on chromosome 8p22 (7). Evidence of linkage to this region has also been found by another group (8), although not by other linkage searches (4). Initially it was suggested that the *999C>T* mutation, as well as other rare variants, may be implicated in prostate cancer risk (9). More recently, it has been suggested that common haplotypes at *MSR1* may also be associated with risk (25). In this study, we focused only on the *999C>T* mutation because it is predicted to truncate the protein and would likely result in loss of several important MSR1 protein domains. Our analysis does not, therefore, address the question as to whether other *MSR1* variants are associated with prostate cancer risk. It does suggest that the magnitude of any real risk associated with the *999C>T* mutation is smaller than originally thought and, importantly, that the contribution of this mutation to overall prostate cancer susceptibility is minimal given its rarity and the low upper bound of its confidence interval. If we assume a risk ratio of 1.34 and a population prevalence of 0.022, then the population attributable risk percent for prostate cancer in association with this mutation would be 0.74%. Moreover, this mutation cannot account for the positive logarithm of odds scores noted in the region around this gene. The most recent studies of this mutation in *MSR1* are in broad agreement with our findings (10, 11).

One potential explanation for the differences in results of association studies in prostate cancer is the variation in

Table 3. Data used for meta-analysis of association of *MSR1*999C>T* mutation with prostate cancer

Subjects (reference)	Controls			Cases			Crude OR	
	Het	WT	Est (95% CI)	Het	WT	Est (95% CI)	Est (95% CI)*	*P*
Caucasians (9)	1	255	0.39 (0.01, 2.16)	8	309	2.52 (1.10, 4.91)	6.60 (0.87, 294)	0.08
Asbestos workers (9)	5	436	1.13 (0.37, 2.63)	2	26	7.14 (0.88, 23.50)	6.71 (0.61, 43.1)	0.12
African Americans (9)	0	110	0.00 (0.00, 3.30)	0	48	0.00 (0.00, 7.40)	—	—
African Americans (10)	0	340	0.00 (0.00, 1.08)	1	133	0.75 (0.02, 4.09)	—	—
Caucasians (11)	16	476	3.25 (1.87, 5.23)	14	482	2.82 (1.55, 4.69)	0.86 (0.39, 1.91)	0.84
Finns (12)	5	475	1.04 (0.34, 2.41)	9	648	1.37 (0.63, 2.58)	1.32 (0.39, 5.04)	0.83
Australians (this study)	50	2,322	2.11 (1.57, 2.77)	39	1,257	3.01 (2.15-4.09)	1.44 (0.92, 2.25)	0.12
<56 y, United Kingdom (this study)	5	181	2.69 (0.88, 6.16)	6	251	2.33 (0.86, 5.01)	0.87 (0.22, 3.64)	>0.9
Ashkenazi Jews, Canada (this study)	3	130	2.26 (0.47, 6.45)	4	129	3.01 (0.83, 7.52)	1.34 (0.22, 9.34)	>0.9

Abbreviations: Het, heterogeneity; Est, estimate; WT, wild-type.
*Estimated using the exact method.

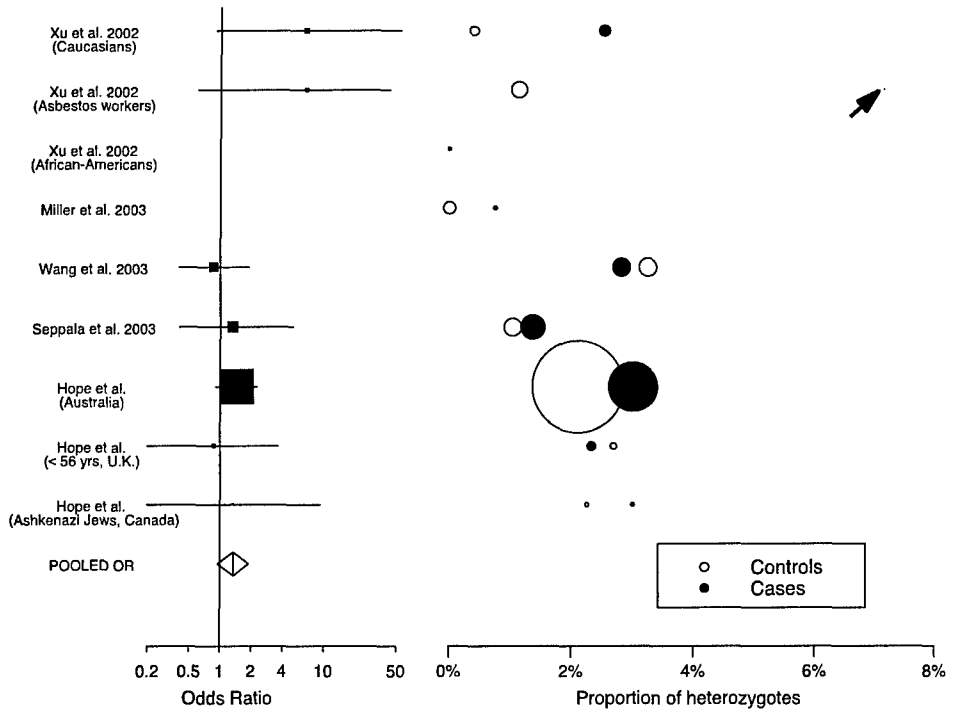

Figure 1. Pictorial representation of results of meta-analysis of previous studies of *MSR1*999C>T* allele frequency and prostate cancer risk. Published *MSR1*999C>T* mutation genotype frequencies for case patients with prostate cancer and for control subjects. Three separate subcomponents of the current study are included. The areas of the symbols are proportional to the size of study. *Left*, odds ratios with 95% CIs; *right*, proportion of heterozygotes in cases (●) and controls (○). For Xu et al. (African Americans; ref. 9), the case and control frequencies (0.00) are superimposed. *OR*, odds ratio.

prevalence of prostate-specific antigen testing, which results in a much higher incidence of disease with low Gleason score. In our study, we included series of cases and controls from countries where the prevalence of prostate-specific antigen screening was high (United States, Canada, where most cases are screen detected), low (United Kingdom, Norway) and intermediate (Australia). We found no evidence of any differences in *MSR1* genotype frequencies in cases or risk ratios between countries, suggesting that prostate-specific antigen screening is not an explanation for the differences.

In the Australian case-cohort series, the controls were, on average, 7 years younger than the cases. A similar age difference was seen for the UK unselected cases and controls. If some of these controls were later destined to become cases within the next 10 years or so, it is possible that we may have underestimated the magnitude of the association between the *999C>T* variant and prostate cancer risk in this series. However, the Australian case-control series was frequency matched on age, and 3.3% of the cases and 2.3% of the controls carried the variant. Moreover, because the risk of prostate cancer between the ages of 55 and 65 is approximately 1% in the populations we studied, we can assume that not more than 1% of the controls are misclassified. This would result in an underestimation of the odds ratio by <0.01, and such an error would not show in two decimal places.

Some groups of prostate cancer cases may be particularly likely to carry the *999C>T* mutation, but it is not possible to identify whether such groups exist from inspection of the data provided here and in the meta-analysis. In particular, there is no evidence that either multiple-case families or early-onset cases are more likely to harbor this mutation (Tables 1 and 3).

Our analysis illustrates a problem inherent in studying so-called "low-penetrance" variants. By definition, they are not associated with large risks. If the population frequency of the supposedly at-risk genotype is very low (e.g., <2%) then very large studies will be required to detect such risks or do so with much precision. As has been shown in a recent meta-analysis of the $Ser^{217}Leu$ and $Ala^{541}Thr$ polymorphisms in the putative prostate cancer susceptibility gene *ELAC2*, it is often the studies with the largest sample sizes that find risk estimates closest to unity (15). This observation is supported by a formal analysis of 55 meta-analyses (26). In most meta-analyses studied, the largest study within each meta-analysis provided more conservative findings than were suggested by the overall result of the meta-analysis. This was true even if the result of the meta-analysis was itself statistically significant. Indeed, the first study published may be inherently likely to overestimate the effect size, sometimes by a considerable margin. This seems to be the case for both linkage (27) and association studies (28). These observations have limited the widespread acceptance of results from smaller series. Notably, as stated by Göring et al. (27), "joint estimation of locus position and effect simply does not work on the same data set, at least when power is ... low...." As has been proposed by the International Collaborative Group for Prostate Cancer Genetics, large meta-analyses of existing data sets may be required to resolve these issues.

Progress in the genetics of prostate cancer susceptibility has been limited by the lack of universally accepted risk-associated genes. Thus far, only mutations in *BRCA2* can be said to be clearly associated with a marked increase in prostate cancer risk (17), although occasional families with apparently disease-associated mutations in other genes such *RNASEL* and *ELAC2* have been reported (5). There have been numerous genome-wide screens completed, with multiple regions of interest identified, but, thus far, none have led to the clear identification of a prostate cancer susceptibility locus (4). This is probably a consequence of considerable genetic heterogeneity, given that recent segregation analyses of prostate cancer favor multigene rather than a single gene model for susceptibility (6).

In conclusion, this very large analysis of case and control series from several countries found no evidence that the *MSR1*999C>T* mutation is associated with increased risk of prostate cancer. When combined with data from all published studies, a meta-analysis found, at best, marginal evidence that the mutation is associated with any risk and that it is unlikely this mutation confers more than a 2-fold increased risk of prostate cancer.

Acknowledgments

We thank all the patients and their families for their participation in this study, the individuals who donated blood (controls; these individuals were identified by Penny Kelham and Artitaya Lophatananon, University of Nottingham), Sheila Seal and Anita Hall for kindly storing the samples that were provided, and Margaret Stevens for database management. We thank Kimberly Kotar and McGill University urologists for recruiting cases in Montreal.

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